



Technical Report

Channel catfish, *Ictalurus punctatus* Rafinesque 1818, tetraspanin membrane protein family: Characterization and expression analysis of CD81 cDNA

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ABSTRACT

CD81, also known as the target of an antiproliferative antibody 1 (TAPA-1) in human, is a member of tetraspanin integral membrane protein family. This protein plays many important roles in immune and other physiological functions. In this report, we characterized and analyzed expression of the channel catfish CD81 transcript. The full-length of channel catfish CD81 cDNA comprised of 1130 nucleotides, including an open reading frame which appears to encode a putative peptide of 234 amino acid residues. By comparison with the human counterpart, the channel catfish CD81 peptide could be divided into domains, including four transmembrane domains, three intracellular domains, and one of each small and large extracellular loops. The degree of conservation of the channel catfish CD81 amino acid sequence to that of mammalian counterparts ranged from 65% to 67%. The large extracellular domain shows the least conservation between fish and mammals. However, the characteristic Cys¹⁵⁹-Cys¹⁶⁰-Gly¹⁶¹ motif and Cys^{176/188} in this domain were conserved. The channel catfish CD81 transcript was detected by RT-PCR in spleen, head kidney, liver, intestine, skin and gill. This result provides important information for further elucidating CD81 functions in channel catfish.

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Channel catfish production is the most important aquacultural industry in the southeastern U.S., generating over 450 million dollars in value annually (USDA, 2007). During studies on the pathogenesis of *Edwardsiella ictaluri*, we found that a battery of channel catfish gene transcripts is up-regulated at the early stage of infection (unpublished data). One of these transcripts is CD81.

CD81, also known as the target of antiproliferative antibody 1 (TAPA-1) and a member of the transpanin integral membrane protein family (Hemler, 2005; Berditchevski and Odintsova, 2007; Levy and Shoham,

2005a,b), was first identified, cloned and characterized in a human lymphoma cell line (Oren et al., 1990). CD81 plays many important roles in immunological and pathophysiological processes in host. CD81 often associated with CD19 is required for humoral immune response to antigens (Maecker and Levy, 1997; Miyazaki et al., 1997; Tsitsikov et al., 1997; Shoham et al., 2003), which event needs CD81 be palmitoylated for lipid raft-dependent receptor signaling (Cherukuri et al., 2004a,b; Clark et al., 2004). After that, the CD81/CD19 complexes associate with the complement receptor CD21 to activate B cells (Fearon and Carroll, 2000; Levy and Shoham, 2005a). Another study demonstrated that CD81 has been dynamically redistributed at the central zone of T-B cell immune synapses, indicating CD81 is involved

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	<-Intracellular->	Transmembrane	>=<-Small Extracellular
	Domain	Region 1	
Human	MG--VEGCTKCIKYLLFVFNFWLGGVILGVALWLRHDPQTNNLLYLE		48
Rhesus monkey	MG--VEGCTKCIKYLLFVFNFWLGGVILGVALWLRHDPQTNNLLYLE		48
Cotton-top tamarin	MG--VEGCTKCIKYLLFVFNFWLGGVILGVALWLRHDPQTNNLLYLE		48
Chinese tree shrew	MG--VEGCTKCIKYLLFVFNFWLGGVILGVALWLRHDPQTNNLLYLE		48
Cattle	MG--VEGCTKCIKYLLFVFNFWLGGVILGVALWLRHDPQTNNLLYLE		48
Pig	MG--VEGCTKCIKDLLFVFNFWLGGVILGVALWLRHDPQTTSLLYLE		48
Mouse	MG--VEGCTKCIKYLLFVFNFWLGGVILGVALWLRHDPQTTSLLYLE		48
Rat	MG--VEGCTKCIKYLLFVFNFWLGGVILGVALWLRHDPQTTSLLYLE		48
African clawed frog	MG--VEGCTKCIKYLLFIFNFIWLGGVILGVALWLRHDPQTSNLLFQQ		48
Channel catfish	MG--VEGCTKCIKYMLFFNFIFWLAGCVILGVSLWLRHDEKTSLLLAK		48
Zebrafish	MVGVEGCTKCIKYMLFFNFIFWLAGCVILGVSLWLRHDTKTSSLLDLK		50
Spotted green pufferfish	MA--VAGCTKCIKYMLFFNFIFWLGGVILGVALWLRHDSQTKGLLIQ		48
	* * *	*	*
		Intracellular Domain	
	Loop	-><- Transmembrane -><- ↓-><-	
		Region 2	
Human	LGDKPAPNTFYVGIYILIAVGAVMMFVGFGLGCYGAIQESQCCLLTGTFFTCL		98
Rhesus monkey	LGDKPAPNTFYVGIYILIAVGAVMMFVGFGLGCYGAIQESQCCLLTGTFFTCL		98
Cotton-top tamarin	LGDKPAPNTFYVGIYILIAVGAVMMFVGFGLGCYGAIQESQCCLLTGTFFTCL		98
Chinese tree shrew	LGDRPAPNTFYVGIYILIAVGAVMMFVGFGLGCYGAIQESQCCLLTGTFFTCL		98
Cattle	LGDRPAPNTFYVGIYILIAVGAVMMFVGFGLGCYGAIQESQCCLLTGTFFTCL		98
Pig	LGDKPAPNTFYVGIYILIAVGAVMMFVGFGLGCYGAIQESQCCLLTGTFFTCL		98
Mouse	LGNKPAPNTFYVGIYILIAVGAVMMFVGFGLGCYGAIQESQCCLLTGTFFTCL		98
Rat	LGDKPAPSTFYVGIYILIAVGAVMMFVGFGLGCYGAIQESQCCLLTGTFFTCL		98
African clawed frog	FEDKQAPGTIFYIGVYIIIAVGAVMMFVGFGLGCYGAIQESQCCLLTGTFACL		98
Channel catfish	FEGTEAPNTFYISVYILIAVGAVMMFVGFGLGCYGAIQESQCCLLTGTFACL		98
Zebrafish	YEGTSPPTFYISVYILIAVGAVMMFVGFGLGCYGAIQESQCCLLTGTFACL		100
Spotted green pufferfish	FEGQQAPGTIFYISVYILIAVGAVMMLVGFGLGCYGAIQESQCCLLTGTFFFFL		98
	* * *	** *	*
	Transmembrane -><-		
	Region 3		
Human	VILFACEVAAGIWGFVNKDQIAKDVVKQFYDQALQQAVVDDAN---NAKA		145
Rhesus monkey	VILFACEVAAGIWGFVNKDQIAKDVVKQFYDQALQQAVVDDAN---NAKA		145
Cotton-top tamarin	VILFACEVAAGIWGFVNKDQIAKDVVKQFYDQALQQAVVDDAN---NAKA		145
Chinese tree shrew	VILFACEVAAGIWGFVNKDQIAKDVVKQFYDQALQQAVVDDEAN---NAKA		145
Cattle	VILFACEVAAGIWGFVNKDQIAKDVVKQFYDQALQQAVVDDAN---NAKA		145
Pig	VILFACEVAAGIWGFVNKDQIAKDVVKQFYDQALQQAVVDDAN---NAKA		145
Mouse	VILFACEVAAGIWGFVNKDQIAKDVVKQFYDQALQQAVMDDAN---NAKA		145
Rat	VILFACEVAAGIWGFVNKDQIAKDVVKQFYDQALQQAVMDDAN---NAKA		145
African clawed frog	VILFACEVAAGIWGFVNKDQISKEMRLFSEVYQHATTGTKEQQ-KALP		147
Channel catfish	VILFACEVAAGIWIHKDQISKDVIGFYDTVYDRGLQETIABKEAAAAA		148
Zebrafish	VLLFACEVAAGIWGMNKDKISKKEVIGFYDSVYDKGATYN-TDNKNPATA		149
Spotted green pufferfish	VILFACEVAAAIWGMNRDITISKELINFYDSAYIKAVDVSGPSKDAAIK		148
	* *	*	*
		Large Extracellular Loop	
Human	VVKTFHETLDCCGSSTLTALTTSVLKNNLCPSGSNIISNLFKEDCHQKID		195
Rhesus monkey	VVKTFHETLDCCGSSTLAALTTSVLKNNLCPSGSNIISNLKKDCHQKID		195
Cotton-top tamarin	VVKTFHETLNCCGSSTLTALTTSVLKNNLCPSGSNISNLFKEDCHQKID		195
Chinese tree shrew	VVKTFHETLNCCSGTLTTLTTSVLKNNLCPSGSNVISNLFKEDCHQKID		195
Cattle	VVKTFHETLNCCGSNTLMTLTTSVLKNSLCPSSGNVITNLFKEDCHGKID		195
Pig	VVKTFHETLNCCGSNTLTTLTTSVLKNSLCPSSGNIISNLMKEDCHSKID		195
Mouse	VVKTFHETLNCCGSNALTTTLTTLRNSLCPSSGNILTPLLPQDCHQKID		195
Rat	VVKTFHETLNCCGSNTLTTLTTLTVLRNSLCPSSSNSFTQLLKEDCHQKID		195
African clawed frog	VLKAFHETLQCCG-DSSSKLSFLNLS-EVCPKRNDILEQTNIEDCHQKID		195
Channel catfish	VLKFPHESLQCCGKGQ---ITSVIS-WATNLCSEPNNLLKNPDCHTFKIK		193
Zebrafish	VLKVPHETLQCCGKGN---LFTAIVDRWLTDTTCPE-HLRTNAVDCHEIK		195
Spotted green pufferfish	ILDFAHSTLDCCGKGDDTALFQQLAGTLCPRKTPEDFLKSQS---CHKLIK		196
	** *	*	*

Fig. 1. Multiple alignments of CD81 amino acid sequences from various species. Gaps introduced in the sequences are indicated as (-). Structural domains of CD81 are indicated above the sequences. Identical amino acids among species are denoted by asterisks (*) below the sequences. GenBank accession numbers of each sequence are as follows: African clawed frog, NP_001080082; cattle, NP_001030271; channel catfish, FJ205473; Chinese tree shrew, ABQ52430; cotton-top tamarin, Q9N0J9; human, NP_004347; mouse, NP_598416; pig, NP_001072147; rat, AAH60583; rhesus monkey, XP_001093228; spotted green pufferfish, CAG05519; and zebrafish, NP_571593.

	-><-	Transmembrane Region 4	-><-	Intra- -> cellular Domain
Human		DLFSGKLYLIGIAAIVVAVIMIFEMILSMVLCCGIRNSSVY		236
Rhesus monkey		ELFSGKLYLIGIAAIVVAVIMIFEMILSMVLCCGIRNSSVY		236
Cotton-top tamarin		ELFSGKLYLIGIAAIVVAVIMIFEMILSMVLCCGIRNSSVY		236
Chinese tree shrew		DLFSGKLYLIGIAAIVVAVIMIFEMILSMVLCCGTRNSSVY		236
Cattle		ELFSGKLYLIGIAAIVVAVIMIFEMILSMVLCCGIRNSSVY		236
Pig		ELFSGKLYLIGIAAIVVAVIMIFEMILSMVLCCGIRNSSVY		236
Mouse		ELFSGKLYLIGIAAIVVAVIMIFEMILSMVLCCGIRNSSVY		236
Rat		ELFSGKLYLIGIAAIVVAVIMIFEMILSMVLCCGIRNSSVY		236
African clawed frog		ALFSTKLYLVGIAAVVAVIMIEMILSMVLCCGIRIYSVY		236
Channel catfish		ELFNDKIYLIGIAALVVAVIMIFEMIFSMVLCCGIRNSPVY		234
Zebrafish		NLFTDKISLIGIAALVVAVIMIFEMIFSMVLCCGIRNSPVY		236
Spotted green pufferfish		ELFSEKLHLIGLALVVAVIMIFEMIFTMVLCCGIRNSP--		235
	**	* * * * *	**	* * * * *

Fig. 1. (Continued).

in the T–B lymphocyte collaboration required for the cell activation (Mittelbrunn et al., 2002). On the other hand, CD81 has been identified as receptors for two important human pathogens. Silvie et al. (2003) demonstrated that CD81 on the cell surface of hepatocytes is required for *Plasmodium* sporozoite infectivity. CD81 is an entry coreceptor on the cell surface of hepatocytes for the hepatitis C virus envelope protein E2 (Cormier et al., 2004; Pileri et al., 1998).

In teleost fish, the zebrafish (*Danio rerio*) CD81 gene has been mapped to LG7 (Yoder and Litman, 2000), but its immunological/pathophysiological functions have not been explored. In the course of studying pathogenesis of *E. ictaluri* in channel catfish, we observed that CD81 expressed sequence tag (EST) was up-regulated at the early stage of infection (unpublished data). This observation prompted us to speculate that CD81 may play a role in early stages of *E. ictaluri* infection. In this report, we describe the isolation, characterization and analysis of expression of the channel catfish CD81 transcript.

The NWAC 103 strain of channel catfish was used in this study as per the Guidelines for the Use of Fish in Research (Nickum et al., 2004). The protocol of animal use was approved by the Institutional Animal Care and Use Committee, Aquatic Animal Health Research Unit, Agricultural Research Service, U.S. Department of Agriculture in Auburn, AL. Tissues were aseptically excised.

Total RNA from tissues was isolated by using a Tri reagent (Molecular Research Center, Inc., Cincinnati, OH) as described previously (Yeh and Klesius, 2008a,b). After total RNA isolation, channel catfish CD81 cDNA was generated by rapid amplification of cDNA ends (RACE) by using a GeneRacer kit (Invitrogen, Carlsbad, CA) per manufacturer's instructions. Primers for PCR amplification are as follows: GeneRacer 5' primer (Invitrogen), 5'-CGACTGGAGCAGGAGCACTGA-3'; GeneRacer 3' primer (Invitrogen), 5'-GCTGTCAACGATACGCTACGTAACG-3'; CD81-45F, 5'-TGCCTGCCTGGTCATCTGTTTGCAT-3'; and CD81-203R, 5'-GCAGCCGAGCCTCTTCTGTCTGA-3'. The PCR products were ligated into the pSC-A cloning vector (Agilent Technologies, Santa Clara, CA). The ligated plas-

mids were transformed into *Escherichia coli* by heat-shock. After culture enrichment at 37 °C in SOC medium, the cells were streaked on LB plates containing 30 µg/ml of kanamycin and incubated at 37 °C overnight. Colonies were randomly picked and cultured in WU medium. No reverse transcriptase added in reactions was included in experiments to ensure that no amplification was from residual genomic DNA.

The DNA sequencing reactions were carried out at the USDA ARS MidSouth Area Genomics Laboratory with an ABI 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA) as described previously (Yeh and Klesius, 2008a,b). More than six clones of each PCR product were sequenced on both strands. Chromatograms were edited and trimmed to remove the vector sequences by using Phred and Lucy software, respectively (Ewing and Green, 1998; Li and Chou, 2004; Ewing et al., 1998). Amino acid sequences were deduced from nucleic acid sequences by using Transeq software (Rice et al., 2000) and aligned with other CD81 amino acid sequences by using ClustalW2 (Larkin et al., 2007) (<http://www.ebi.ac.uk/services/index.html>). Expasy server (Gasteiger et al., 2005) was used to calculate the CD81 molecular mass and pI. Transmembrane topology and signal peptide of the channel catfish CD81 peptide were predicted via the Phobius web server (Käll et al., 2007). Phylogenetic relationships of CD81 from various species were analyzed by the MEGA 4.0 software (Tamura et al., 2007) based on the ClustalW2 alignment results.

RT-PCR assays for CD81 gene transcript in channel catfish tissues were performed by a two-step procedure routinely used in our laboratory (Yeh and Klesius, 2008a,b). Primers (CD81-45F and CD81-203R) were also used in these assays. Primers for β-actin were β-actin F, 5'-GACTTCGAGCAGGAGATGGG-3' and β-actin R, 5'-AACCTCTCATTGCCAATGGTG-3'. These amplified products were analyzed in 2% agarose gel electrophoresis and stained with ethidium bromide. Images were recorded by a KODAK Gel Logic 440 Imaging System and processed with Adobe Photoshop (v. 7.0.1., Adobe Systems Incorporated, San Jose, CA).

We previously identified a channel catfish CD81 expressed sequence tag by subtractive suppression

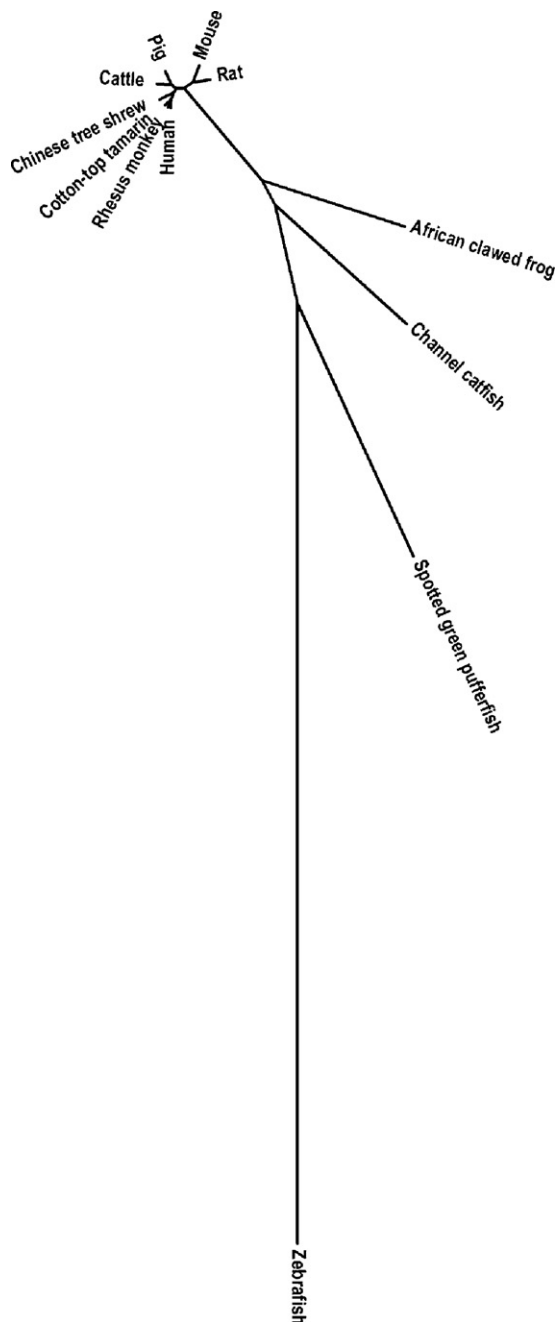


Fig. 2. Molecular phylogenetic relationships of CD81 amino acid sequences among species. The sequences from Fig. 1 were used for the phylogenetic tree generation by bootstrap analysis (1000 replications) in MEGA4 software (Tamura et al., 2007).

hybridization (unpublished data). Based on this EST, we continued to clone, sequence and characterize the channel catfish CD81 cDNA with the RACE method (Frohman et al., 1988). The full-length of the channel catfish CD81 cDNA had 1130 nucleotides (GenBank accession numbers FJ205473), including 5'- and 3'-untranslated region (UTR), and an open reading frame

(ORF). The ORF appears to encode a 234-amino acid peptide with a calculated molecular mass of 25,993 Da and *pI* of 6.09. The 5'-UTR contained a TATA box sequence (TATAAA) at positions –39 to –34, and a Kozak sequence (ACCATGG) at positions –3 to +4 upstream of the translation start codon. The 3'-UTR had a polyadenylation tail. Recently, similar EST of channel catfish CD81 was deposited in the GenBank's EST database (www.ncbi.nlm.gov/dbEST/).

We further analyzed the deduced CD81 amino acid sequence, and we found that, like the mammalian counterparts (Hemler, 2005; Levy and Shoham, 2005a,b), channel catfish CD81 is a transmembrane protein, which can be structurally divided into four transmembrane domains, three intracellular domains and two (small and large) extracellular loops (Fig. 1). Unlike among mammals shared more than 85% homology (Cho et al., 2007), the deduced channel catfish CD81 amino acid sequence shared 79% identity with zebrafish, and 65–67% identity with the mammals. In zebrafish, Yoder and Litman (2000) also demonstrated that the CD81 peptide of zebrafish is 66% and 65% identical to that of human and mouse, respectively. As shown in Fig. 1, we observed that although the large extracellular loops showed the least conservation between fish and mammals, the characteristic Cys¹⁵⁹–Cys¹⁶⁰–Gly¹⁶¹ motif and Cys^{176/188} (numbering after channel catfish) (Kitadokoro et al., 2001) of the large extracellular loops among species examined were conserved, suggesting that the three-dimensional structure of the large extracellular loop of CD81 may be conserved via disulfide linkages throughout the evolution (Fletcher et al., 1994; Rushmere et al., 1994). Other key conserved feature includes the potential palmitoylation at the Cys⁶–Thr⁷–Lys⁸–Cys⁹ motif and at the Cys²²⁵–Cys²²⁶ sites in intracellular domains.

A phylogenetic tree was generated using the ClustalW2 alignment results (Fig. 1). As seen in Fig. 2, mammalian CD81 formed a very closely supported clade, distinguishable from that of fish counterparts, which are heterogeneous groups of over 27,300 species (Helfman, 2007). These results are in agreement with our previous findings in other channel catfish genes (Yeh and Klesius, 2007a,b, 2008a,b,c).

The expression profile of channel catfish CD81 was examined in spleen, head kidney, liver, intestine, skin and gill with multiplex RT-PCR amplification. The amplified CD81 and β -actin products had 159 and 203 nucleotides, respectively. As seen in Fig. 3, the channel catfish CD81 transcript was detected in all tissues of fish examined. These results are in agreement with reports for the mammalian counterparts that CD81 is ubiquitous on animal cell surfaces (Hemler, 2005; Levy and Shoham, 2005a).

In summary, the channel catfish CD81 cDNA transcript was cloned, sequenced, and characterized. The transcript was constitutively expressed in all tissues examined. Experiments for the CD81 expression in *E. coli* and production of polyclonal antisera that will be used to further explore the channel catfish CD81 functions are underway.

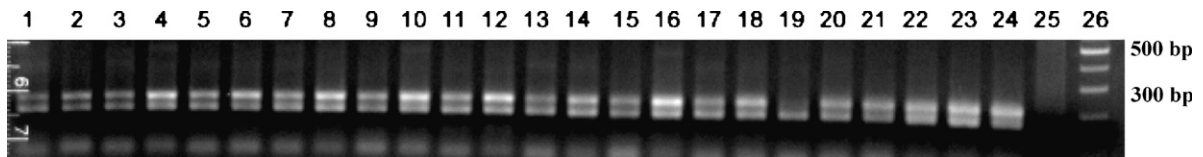


Fig. 3. Tissue distribution of channel catfish CD81 transcript ($n = 4$). Total RNA from various tissues was used for RT-PCR assays (Yeh and Klesius, 2008a,b). The amplified products were analyzed by agarose gel electrophoresis and stained with ethidium bromide. The sizes of amplified CD81 and β -actin were 159 and 203 nucleotides, respectively. Spleen (lanes 1, 7, 13, and 19), head kidney (lanes 2, 8, 14, and 20), liver (lanes 3, 9, 15, and 21), intestine (lanes 4, 10, 16, and 22), skin (lanes 5, 11, 17, and 23), and gill (lanes 6, 12, 18, and 24). Lane 25, negative control and lane 26, 100 bp molecular weight markers (500, 400, 300 and 200 nucleotides).

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